1647

MPG-8

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Examiner	:	Sandra L. Wegert
Group Art Unit	:	1647
Applicant(s)	:	Pardo-Fernandez et al.
Application No.	:	09/694,777
Confirmation No.	:	8515
Filed	:	October 23, 2000
For	:	NOVEL HUMAN K^+ ION CHANNEL AND THERAPEUTIC APPLICATIONS THEREOF

New York, New York
March 25, 2002

Hon. Commissioner for Patents
Washington, D.C. 20231

TRANSMITTAL LETTER

Sir:

Applicants filed a copy of the Declaration of Dr. Luis A. Pardo-Fernandez under 37 C.F.R. §1.132 and Exhibits A-G with applicants' March 19, 2002 Response. For the convenience of the Examiner, applicants submit herewith clearer copies of those documents.

Applicants believe that no fees are associated with this submission. However, if fees are due, the Director is authorized to charge payment of any fees to Deposit Account No. 06-1075. A duplicate copy of this transmittal letter is enclosed herewith.

Respectfully submitted,

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I Hereby Certify that this Correspondence is being Deposited with the U.S. Postal Service as First Class Mail in an Envelope Addressed to: ASSISTANT COMMISSIONER FOR PATENTS P.O. Box 2327 Arlington, VA 22202 on

March 25, 2002

Claire [illegible]

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DECLARATION OF DR. LUIS A. PARDO-FERNANDEZ UNDER 37 C.F.R. ' 1.132

I, Luis A. Pardo-Fernandez, a citizen of Spain, residing at Hermann–Rein–Strasse 3E, Göttingen, Germany, hereby declare that:

1. I am an inventor of the above-identified application.

2. I received an M.D. (honors) degree in from University of Oviedo (Spain) in 1986. I received a Ph.D. degree in Biochemistry and Molecular Biology in 1990 from University Oveido (Spain). I have given approximately nine invited seminars and published approximately twenty papers in the area of ion channels and receptors.

3. I have been engaged in the study of neuroscience and, in particular, ion channels, for 10 years. From 1996–2001, I was employed by the Max-Planck-Gesellschaft, Max-Planck-Institute for Experimental Medicine as Staff researcher and head of the EAG group. In August 2001, I was appointed Chief Scientific Officer of iOnGen, of Göttingen, Germany, a position which I hold presently. I attach a copy of my *curriculum vitae* as "Exhibit A."

4. I have read the December 19, 2001 Office Action in the above-identified application. I understand that the Office Action includes a rejection of claim 15 under 35 U.S.C. § 112. Specifically, I understand that the Examiner has stated that:

> [T]he subject matter [of claim 15] was not described in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. The specification is not enabling for the limitation of the claims wherein a composition comprising a nucleic acid, polypeptide or antibody is used in diagnosis.
>
> . . .
>
> However, the claim recites use of a composition of nucleic acids, polypeptides and/or antibodies for "diagnosis". There is no enabling discussion or working examples disclosed in the instant application as to how or what disease is related to the eag K^+ channel disclosed in the specification, nor is there discussion of how one would practice the method of diagnosing a disease.

5. I make this declaration to affirm that the specification provides guidance sufficient to use the nucleic acids, polypeptides and antibodies of this invention to diagnose a condition, and to provide the experiments demonstrating the diagnosis of conditions using the methods and diagnostic compositions of this invention.

6. I note that the application states that diagnostic compositions are useful in detecting the onset or progress of diseases related to the undesired expression or overexpression of the nucleic acid molecule of the invention (e.g., page 20, lines 18–20 of the

increased or ongoing cellular proliferation (e.g., page 20, lines 20–21 of the specification as filed). The application more specifically teaches that methods of this invention can be used to diagnose cancer, neurodegenerative diseases, or psoriasis (e.g., page 27, lines 1–8 specification as filed). Further, the application teaches that the diagnostic composition comprises the nucleic acid molecule of the invention, the vector of invention, the polypeptide of the invention and/or the antibody of the invention (page 20, lines 15–17 of the specification as filed).

7. I note that the application states that suitable protocols for carrying out the methods of the invention are well-known in the art and include, Northern blotting for the assessment of the level of mRNA or the analysis of tissue by microscopic techniques using antibodies that specifically recognize the polypeptide of the invention (page 27, lines 10–13). Further, the application exemplifies the use of techniques known in the art, such as RT-PCR, to evaluate the expression of the *EAG* of this invention in diseased or normal cells or tissues (e.g., page 31, lines 15–19; Figure 15; Example 2). Accordingly, one of ordinary skill in the art could diagnose a disease according to this invention using the diagnostic and the methods of the application.

8. In further support, I provide data obtained by using diagnostic compositions of this invention in methods for diagnosing cancer. Briefly, I followed the teachings of the specification by using techniques recited in the specification in combination with nucleic acid molecules derived from the *EAG* sequence of the invention and antibodies directed against the EAG protein to detect differential expression of EAG in normal tissue or tissue obtained from primary tumor biopsies. The results of the use of the two techniques, RT-PCR and immunohistochemistry, are presented below.

9. My colleagues and I performed RT-PCR assays that measured levels of *EAG* and transferrin receptor (*TRF*) RNA expression in breast tissue or brain tissue obtained from normal patients or breast tissue from primary tumor biopsies. The *TRF* RNA measurements were used to normalize the *EAG* RNA measurements based on the quantity of mRNA retrieved from the test tissue samples.

10. We prepared cDNA from normal brain and breast tissue and breast tumor tissue from biopsies. For most normal tissues, we purchased total RNA from normal tissues from Clontech Laboratories GmbH (Heidelberg, Germany). We prepared total RNAs from tumor biopsies using the RNeasy kit from Qiagen GmbH (Hilden, Germany). Next, we reverse transcribed the total RNAs into cDNAs using SuperScript reverse transcriptase (Invitrogen, Karlsruhe, Germany). Details for protocols for RNA purification, reverse transcriptase reactions and TaqMan PCR are attached hereto as Exhibit B.

11. We prepared cDNA from synthesized *EAG* samples. Briefly, we inserted the nucleic acid sequence of human *EAG* cDNA into the pSGEM vector. We used the resulting clone to synthesize complementary *EAG* RNA (cRNA) according to a standard protocol (Krieg and Melton. *Meth. Enzymol.* 155, 397–415, 1987). We determined the concentration of the synthesized *EAG* RNA by fluorescent labeling the RNA with RiboGreen reagent (Molecular Probes, Eugene, OR) and comparing the labeled RNA to known concentrations of RNA that have also been labeled with RiboGreen reagent. We next mixed different, known amounts of the synthesized *EAG* RNA with total RNA isolated from rat liver. We then reverse transcribed the mixture of RNA into cDNA for use in generating a standard curve after being subject to the TaqMan PCR described below.

12. We performed real-time TaqMan PCR on the cDNAs derived from the synthetic RNA from paragraph 11 and the sample RNAs from paragraph 10 following the protocol of Higuchi *et al.* (*Bio/Technol.* **11**, 1026–1030, 1993) and using the TaqMan system in an ABI Prism 7700 Sequence Detector (Applied Biosystems, Foster City, CA). In the TaqMan system, there is an inverse correlation between the initial amount of template present in the sample and the number of PCR cycles required to reach a defined threshold value. Accordingly, the TaqMan system in this study was set at the number of PCR cycles optimized to reach the detection threshold for each sample. The diagnostic composition of the TaqMan PCR assay included primers that hybridize to portions of the *EAG* sequence, fluorescently labeled probes that hybridize to portions of the *EAG* sequence and, optionally, primers that hybridize to portions of the *TRF* sequence, fluorescently labeled probes that hybridize to portions of the *TRF* sequence, or cDNAs derived from the synthetic EAG samples, normal tissues or biopsies.

13. The following sequences of h*EAG* primers and probe were used in the Taqman PCR assays with each cDNA sample:

5'-TCTGTCCTGTTTGCCATATGATGT-3'

5'-CGGAGCAGCCGGACAA-3' and

FAM-5'-AACGTGGA-Amino C6 dT-GAGGGCATCAGCAGCCT-3' (probe)

14. The following sequences of *TFR* primers and probe were used in the TaqMan PCR assays with the cDNAs derived from the normal tissues or biopsies:

5'-GACTTTGGATCGGTTGGTGC-3'

5'-CCAAGAACCGCTTTATCCAGAT-3' and

JOE-5'-TGAATGGCTAGAGGGA-TAMRA dT-ACCTTTCGTCCC-3' (probe).

15. Thus, the *EAG* RT-PCR products were labeled with the fluorescent dye, FAM (carboxy-fluorescein), and the *TRF* RT-PCR product were labeled with JOE (carboxy-dimetoxyfluorescein).

16. We used the values of the fluorescent emissions from the RT-PCR products derived from the synthesized *EAG* RNA to generate a standard curve. The standard curve was used to interpolate the concentration of the *EAG* RT-PCR products in the normal tissue or biopsies. We normalized the interpolated values against the amount of *TRF* RT-PCR product observed in each sample.

17. In Exhibit C, we further calculated the *EAG* RNA concentration in the breast cancer biopsies as a ratio relative to the *EAG* RNA concentration in normal human brain tissue as determined by RT-PCR above. Accordingly, the *EAG* RNA content of normal human brain used to produce this Table is scored as 1.0 (not shown).

18. We show in Exhibit C that the *EAG* RNA of this invention is overexpressed in breast cancer tissues compared to tumor-free tissue of breast carcinoma biopsy specimens and normal mammary gland tissue. This data supports the use of the diagnostic compositions of this application in the diagnosis of diseases.

19. As discussed in paragraph 7, *supra*, and page 27, lines 11–13 of the specification, diagnostic compositions comprising anti-EAG antibodies can be used for diagnosing cancer. For example, antibodies can be used to immunohistochemically detect EAG potassium channels in cancerous tissues. My colleagues and I prepared formalin-fixed and paraffin-embedded archival biopsy specimens and purchased multiple tissue arrays

(BioCat, Heidelberg, Germany). Using formalin-fixed and paraffin-embedded tissues of human cerebral cortex, we developed the optimal conditions, for immunostaining the biopsy specimens and tissue arrays using the anti-EAG monoclonal antibody, EAG1.62.mAb. The biopsy specimens and tissue arrays were prepared and contacted with EAG1.62.mAb as described in Exhibit D. The samples were then washed, contacted with a second antibody conjugated to hydrogen peroxidase using the Envision Peroxidase System and exposed to a diamino benzidine tetrahydrochloride solution (DAB; DAKO Diagnostica, Hamburg, Germany) as described in Exhibit D.

20. Antigen expression, that is, EAG protein expression, was presented as an immunoreactive score (IRS). The signal intensity (SI) of the overall slide was given a value from 0 (negative) to 3 (intensive). The staining amount (SA) value was determined by counting the number of cells stained out of the total cells and assigning a value for the percentage of stained cells as follows: 1, 0%–10% stained cells; 2, 11%–50% stained cells; and 3, >50% stained cells. The maximum IRS was calculated by adding the highest SI and SA values for each sample.

21. The monoclonal antibody EAG1.62.mAb clearly detects the preferential expression of EAG protein in tumor cells as compared to normal cells (Exhibit E). This data supports the use of the diagnostic compositions of this application in the diagnosis of diseases.

22. We further analyzed an expanded number of tissues with the diagnostic compositions of this application. Using monoclonal antibody EAG1.62.mAb on tissue samples of various origins, such as multiple tissue arrays and additional biopsy samples, we show that EAG protein overexpression in tumor cells compared to normal tissue is a very

frequent occurrence. This data, shown in Exhibit F, further supports the use of the diagnostic compositions of this application in the diagnosis of diseases.

23. Exhibit G shows representative images of normal and neoplastic tissue that have been immunoperoxidase stained for EAG using a diagnostic composition comprising monoclonal antibody EAG1.62.mAb. Tissue samples from normal mammary gland epithelium (A) show only low levels of EAG expression, which is in contrast to high levels in invasive carcinomas (B). Similarly, normal prostate tissue (C) show low levels of EAG expression whereas prostate carcinoma (D) shows high levels of expression. Normal colon epithelial cells (E) have detectable EAG expression but it is much less than in colon carcinoma (F, from the same colon tissue). In bronchial tissue, EAG expression is undetectable in the slightly hyperplastic epithelium (G), but EAG is highly expressed in squamous cell carcinoma (H). Normal liver (I) does not show detectable levels of EAG expression. In contrast, hepatocellular carcinomas (J) have high levels of EAG expression. The arrowheads in A and G indicate inflammatory cells that stain strongly positive and served as internal positive controls. Thus, we confirmed the observation that the overexpression of EAG protein in cancerous tissue is diagnostic of cancer.

24. Using known techniques such as immunostaining, in combination with a diagnostic composition containing an anti-EAG antibody, we were able to stain many cancerous tissues. This data supports the use of diagnostic compositions of this application in the diagnosis of diseases.

25. I declare further that all statements made herein of my own knowledge are true and that all statements made herein on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001, Title 18, United States Code, and that such willful false statements may jeopardize the validity of this application and any patent issuing thereon.

Luis A. Pardo-Fernandez

Signed this 19th day of March, 2002
at Göttingen, Germany.